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Attachment of Popup Archival Transmitting (PAT) Tags to Loggerhead Sea Turtles (*Caretta caretta*)

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Popup Archival Transmitting (PAT) tags are a relatively new tool used in marine animal studies (e.g., Block et al. 1998; Prince and Goodyear 2006; Sims et al. 2005). These tags differ from conventional satellite tags in that they collect and archive temperature, depth, and light level (for geolocation estimates) data over a period of time and then automatically are released from the animal on a designated date and transmit data to ARGOS satellites; the tag must detach before the data are transmitted. PAT tags are often used in survival studies (Domeier et al. 2003; Horodysky and Graves 2005).

One of the most important components of the system is the tether and how it is attached to the animal to ensure that the tag stays on and does not affect the behavior of the animals. Additionally, attachment is a compromise between the need for the tag to be released properly from the animal and the need for a long-term attachment which would still allow for a break-away link should the animal become entangled in fishing gear, marine debris, etc. Because the animals do not need to surface with the tag so that the satellite receives the data—instead the tag is released, floats to the surface, and transmits the archived data—the tether can be quite short, which reduces the risk of entanglement.

Marine turtles are protected species that interact with numerous types of fishing gear throughout the world (Gerosa and Casale 1999; Henwood and Stuntz 1987; Julian and Beeson 1998), often with lethal consequences. Pelagic longlines are identified as a source of mortality for marine turtles (Kotas et al. 2004; McCracken 2000; Yeung 2001), although the majority of the animals are released alive (Watson et al. 2005; see references above). However, the impacts of the fisheries have yet to be quantified because the post-hooking mortality rates are not known. NOAA Fisheries initiated a project in 2001 to evaluate the feasibility of using PAT tags to determine the post-hooking survival rates of marine turtles interacting with the pelagic longline fishery in the North Atlantic (Epperly et al. 2002; Sasso and Epperly 2007). Herein, we describe the tether system and attachment of PAT tags to Loggerhead Turtles, *Caretta caretta*, used in the pilot study. We monitored the attachment of PAT tags on three captive-reared loggerhead sea turtles in order to (i) evaluate the impact of the attachment on the turtles and (ii) determine if the attachment could remain intact for a one year deployment, the duration we needed to evaluate annual survival rates at sea.

Test Animals.—In May 2003 non-functional, but otherwise intact PAT tags (Wildlife Computers, Inc., Redmond, Washington, USA) were attached to three captive reared Loggerhead Sea Turtles in the NOAA Fisheries Service Laboratory in Galveston, Texas, USA (Fig. 1). The 31 month-old animals hatched from nests adjacent to Clearwater, Florida, USA in 2000. After emergence from the nest, the hatchlings were transferred to the Galveston rearing facility. Husbandry of turtles at this facility is described in Higgins (2003). The animals ranged in size from 48.1 to 49.8 cm minimum straight carapace length (SCL) at the time of tag attachment. In early June, soon after PAT tags were attached, one turtle (RRA816) was transferred to the NOAA Fisheries Service Laboratory in Panama City, Florida, USA where it was placed in a large outdoor pen (14.2 m long × 7 m wide × 0.9–1.5 m deep, depending on the tide) in St. Andrew Bay, with 12 or fewer other individuals of the same age. The other two turtles remained in Galveston. The one turtle was moved to better simulate conditions in the wild. The large outdoor pen allowed the turtle to swim more and faster and to dive deeper, exposed the animal to wave action and to UV radiation, and allowed for interaction with structures and with other animals. While in Panama City the turtle was fed a diet of squid (*Illex illecebrosus*). The Panama City turtle was returned to Galveston in early July. While in Galveston, the turtles were fed a minimal growth diet of whole Atlantic Mackerel (*Scomber scombrus*) in order to simulate the growth rates of wild turtles of that size (~5 cm yr^{-1}) (Mendonça 1981; NOAA Fisheries Service, unpubl. data; Schmid 1995). The turtles were held in-

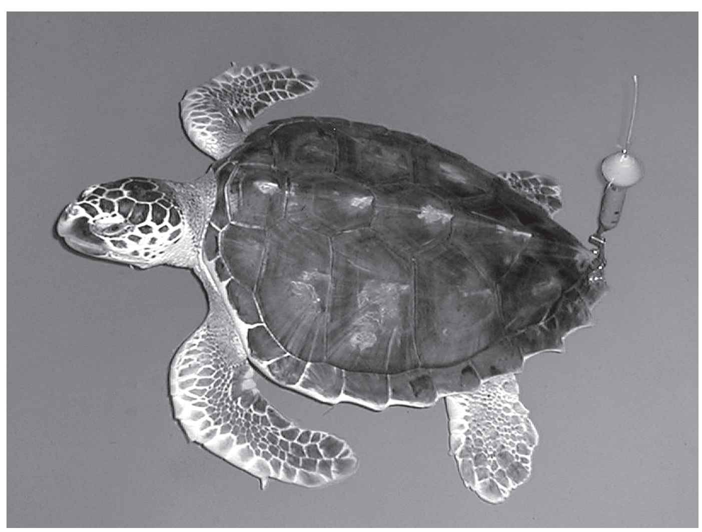

FIG. 1. Popup Archival Transmitting tag (PAT) attached to a captive-reared loggerhead sea turtle (*Caretta caretta*), approximately 49 cm minimum straight carapace length, in a raceway at NOAA Fisheries Service Laboratory in Galveston, Texas, USA.

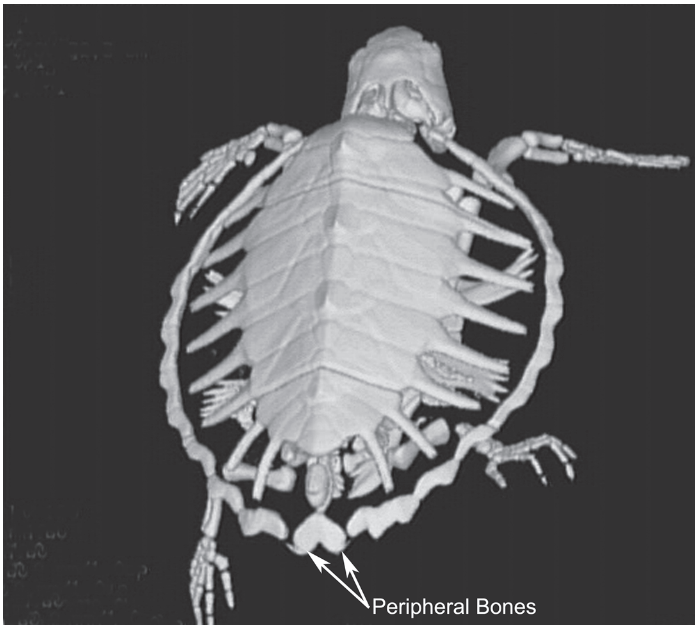

FIG. 2. Computed tomography (CT) scan of an immature loggerhead turtle showing peripheral bones which underlie postcentral scutes.

doors in a large divided raceway where each was allocated a space of 2 m × 1.8 m with a water depth of 0.9 m.

PAT Tag Attachment.—In order to attach PAT tags, the animals were removed from their raceway and immobilized on a platform of clean wet foam placed in a shallow plastic crate. Throughout the process, all attempts were made to maintain as aseptic a working environment as possible. Latex surgical gloves were worn throughout and changed frequently to avoid contamination. The caudal surfaces of the plastron and carapace were cleaned and any barnacles were removed. The area was scrubbed using sterile gauze sponges and 10% povidone iodine solution at least three times over a 15 min period, and the area was maintained damp with the solution. The posterior carapacial scutes were iced for 15 min to numb the area to be drilled. All attachment hardware and the drill bit were soaked in 10% povidone iodine solution for 15 min before they came in contact with the turtle. A 4.8 mm titanium nitride coated steel drill bit was used and drill speed was low to minimize frictional heat. Although there was no bleeding, a drop of Clotisol® (Benepet® Pet Care Products, St. Joseph, Missouri, USA; ferric sulfate, aluminum sulfate, collagen protein, and chloroxylenol in suspension) was placed in each hole prophylactically, and then the hole was flushed with 10% povidone iodine solution.

An eye strap connected to the carapace was used as an anchor for attaching the PAT tag (Fig. 1). The attachment involved drilling a pair of holes through the postcentral scutes and their peripheral bones, from dorsal to ventral (Fig. 2 and Fig. 3). The centers of the holes corresponded to centers of the attachment holes in the eyestrap. A 3.8 cm × 1.1 cm 18-8 stainless steel (SS) standard line eye strap was attached to the turtle with 2 SS slotted, round head bolts (#10/24 × 2.4–5.1 cm) and a #10/24 SS locking nut with nylon insert. The bolts were inserted ventral to dorsal when the length of the bolt was long enough to extend well past the locking nut and potentially chafe the rear flippers. In contrast, a dorsal to ventral insertion was used when the bolt was appropriately sized such that it did not extend well past the locking nut. Nylon washers (6.4 mm outside diameter) were placed between the SS hardware and the turtle (Fig. 3). Nylon washers were used rather than SS washers because nylon is both inert and flexible, allowing for fit to a scute's irregular surface, and it does not have any sharp edges. The locking nuts were threaded over exposed bolt ends and tightened until there was no space between the pieces of attachment hardware.

PAT Tag Tether.—A 10 cm long tether was used to connect the PAT tag to the eye strap. One end of the monofilament (182 kg test fluorocarbon, 1.8 mm diameter) was looped around the plastic thimble attached to a corrodible pin in the tag, and crimped using SS sleeves (oval, for 1.6 mm wire rope). The other end was looped around a 2 mm SS thimble for monofilament line and crimped. The thimble was attached to the eye strap before the bolts were

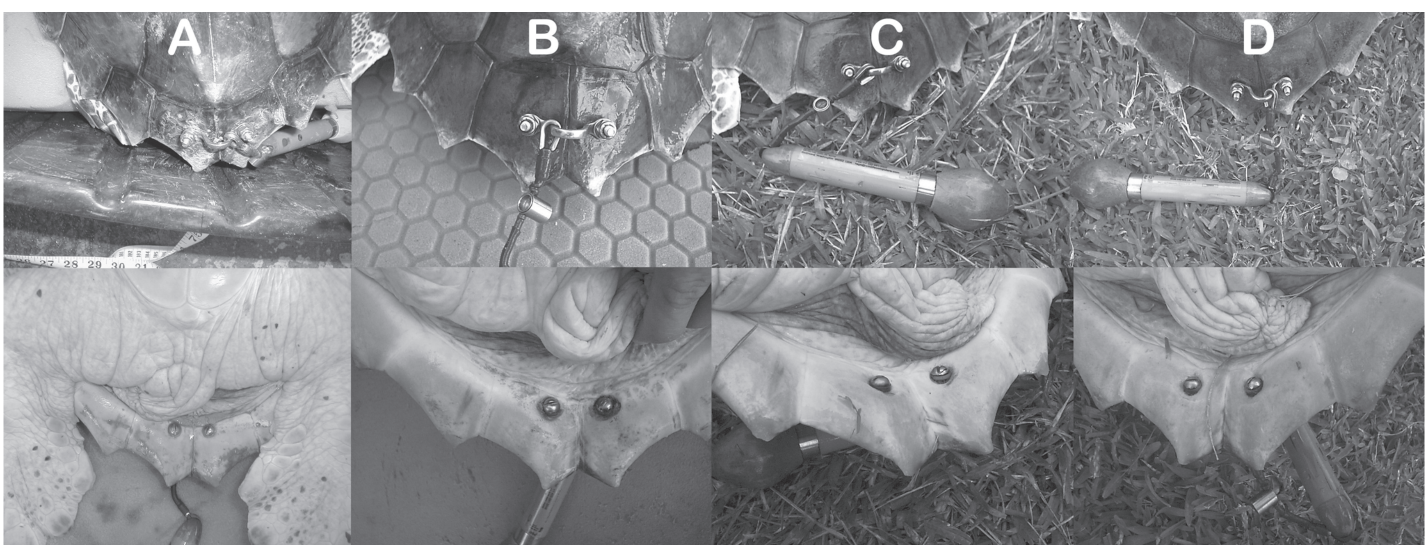

FIG. 3. Dorsal and ventral views of the PAT tag attachment on a 2000 year class loggerhead sea turtle (*Caretta caretta*; RRA806) photographed throughout the year: (A) May 22, 2003, (B) July 24, 2003, (C) November 17, 2003, and (D) May 20, 2004. The eye strap is bolted through the postcentral scutes and the underlying peripheral bones.

threaded through the shell. In order to prevent the tag from being crushed under extreme pressure (usually associated with great depths), the tether was equipped with a device (RD-1500) that severs the monofilament releasing the tag when a depth (approximately 1500 m) well outside the normal diving range of sea turtles is reached. The RD-1500 was centered on the tether. Marine grade adhesive-lined heat shrink tubing (3.2 mm and 4.8 mm inner diameter for line and crimp sleeves, respectively) was applied over the monofilament and both crimp sleeves on either side of the RD-1500 to reduce the monofilament's exposure to ultraviolet radiation and to prevent abrasion of the line. A corrodible pin base of the PAT tag provided a breakaway safety link for the turtle (22.7 kg static weight breaking strength) in case of unanticipated entanglement.

Monitoring.—During the following 12 months, the turtles were monitored for changes to the attachment (e.g., loosening bolts, corrosion), the tether (e.g., chafing, breakage), and impact to the turtle (e.g., necrosis, entanglement). The turtles were removed weekly from the raceways and both the dorsal and ventral surfaces of the attachment were photographed (Fig. 3). All authors monitored the fine resolution digital photographs (1024 × 768 pixels).

Computed Tomography Imagery.—The tags were removed in May 2004 and the animals, now 51.4–56.0 cm SCL (13.4–22.2 kg), were examined using Computed Tomography (CT) imaging (Wyneken 2005) to evaluate the impact of the attachment on the underlying bone (Fig. 4). Turtle health and body condition were normal. Prior to CT imaging, the animals were fasted for more than 48 hours. After removing the hardware, the animals were anesthetized using ketamine (5 - 6 mg/kg IV), medetomidine (0.15–0.18 mg/kg IV), and reversed with atipamezole (0.7–0.9 mg/kg IM) (after Chittick et al. 2002). The animals were sedated in 2–3 min and lightly anesthetized in 4–9 min. Animals were reversed 18–26 minutes after their initial injections, and recovered to a mildly sedated condition in 17–18 min. While sedated, the animals' heart rates were monitored using an ultrasonic doppler flow detector with an 8.9 MHz probe (Model 811-BTS, Parks Medical Electronics, Inc., Aloha, Oregon).

Results and Discussion.—Previous attachment methods for conventional satellite tags on cheloniid turtles relied on epoxy glued bases and/or fiberglass (Balazs et al. 1996; Godley et al. 2002; Swimmer et al. 2006). However, because of the turnover in scute material, these attachment systems may be sloughed before the tracking time is complete. Conventional tags required placement high on the carapace or at the end of a long tether to allow for transmission when a turtle surfaces. The PAT tag does not transmit while attached to the study animal. Thus, we were not restricted to placement high on the carapace, where drag is highest (Watson and Granger 1998), and could use a short tether. We elected to use a through-bolted attachment, relying on a pair of holes, rather than a single hole, to distribute torque from the tether over two attachment points and increase the chance of maintaining attachment integrity. The postcentral scutes were selected because: (i) the rear aspect of animals with tapered body profiles is a region of low drag (after Bannasch et al. 1994; Watson and Granger 1998); (ii) the postcentral scutes are supported by a pair of articulated peripheral bones that provide a strong, accessible and easily located attachment site (Fig. 2) (see Wyneken 2001), and (iii) given a sufficiently short tether length, both it and the tag would be out of the way of the rear flippers and the turtle's visual field. Holes were drilled proximally on the postcentral scutes, but clear of the ventral skin, to ensure that both bone and keratin supported the bolts. If holes are drilled distally in the scutes, they might only pass through the keratin, a weaker support than the composite we chose.

We used two criteria in the design of the tether. First, we wanted the tether to be long enough for tag to reside within the turbulent wake of the turtle, minimizing or effectively eliminating hydrodynamic costs to locomotion. Thus, the tether had enough length (10 cm) to allow the tag to follow behind the turtle and somewhat above the posterior-most part of the carapace. Secondly, the tether was also short enough so that the tag trailed linearly and avoided entangling or bumping the hind limbs when the turtles were swimming or in rough surface waters. The construction of our tether for sea turtles was modeled on the tether used in research of pelagic fishes (Graves et al. 2002; Prince and Goodyear 2006).

No general anesthesia or local anesthetic was administered during attachment. There are no conclusive studies of pain control in turtles or applicable studies in other reptiles to override the need to avoid risks from general anesthesia or injectable mammalian analgesics (Schumacher and Yelen 2005; Wyneken et al. 2005). In order to simulate anticipated field conditions in which the PAT tags would be attached and deployed on wild animals, we used ice as a potential local analgesic and avoided anesthetics. Trained fisheries observers would be attaching the tags to turtles caught at sea, often days to a week from the nearest port and away from veterinary support. Additionally, turtles would be released immediately following PAT tag attachment so post-anesthesia monitoring would not be possible. Furthermore, research permits would not allow for the administration of general anesthesia by the observers. Local anesthetic injection was not feasible as the attachment site was hard keratin overlying bony carapace. Innervation to the shell is via spinal nerves and their extent within bones is unknown. While we made attempts to minimize discomfort (15 min focal ice application, slow drill speed), some turtles initially reacted to the drilling by pushing with their limbs against the foam and crate, attempting to propel themselves forward, and were briefly (< 1 min) restrained. Based on videos of the animals taken immediately after they were placed in their holding tanks and taken the day after attachment, there was no overt evidence of continued discomfort subsequent to the attachment procedure, and weight gain over the next year was within the range for turtles without PAT tag attachment (NOAA Fisheries Service, unpubl. data).

The attachments remained intact for the full year (22 May 2003–25 May 2004) (Fig. 3), but the only grossly visible responses occurred within the first month. Within a week of attachment there was evidence of localized pressure necrosis deep to the washer on the ventral surface. The initial necrosis either progressed slightly or the holes wore over the next several weeks so that at the end of the first month the holes had enlarged a small amount allowing the eye straps to rock slightly (maximum ~1.5 mm) along the turtle's anterior-posterior axis and the bolts could move vertically (1–3 mm); these changes did not progress further. The nuts held fast and never loosened. The greatest eye strap movement was observed in the one turtle that was moved temporarily to a large outdoor pen in Panama City, Florida where it could swim greater distances and interact with other turtles as well as with the pen's

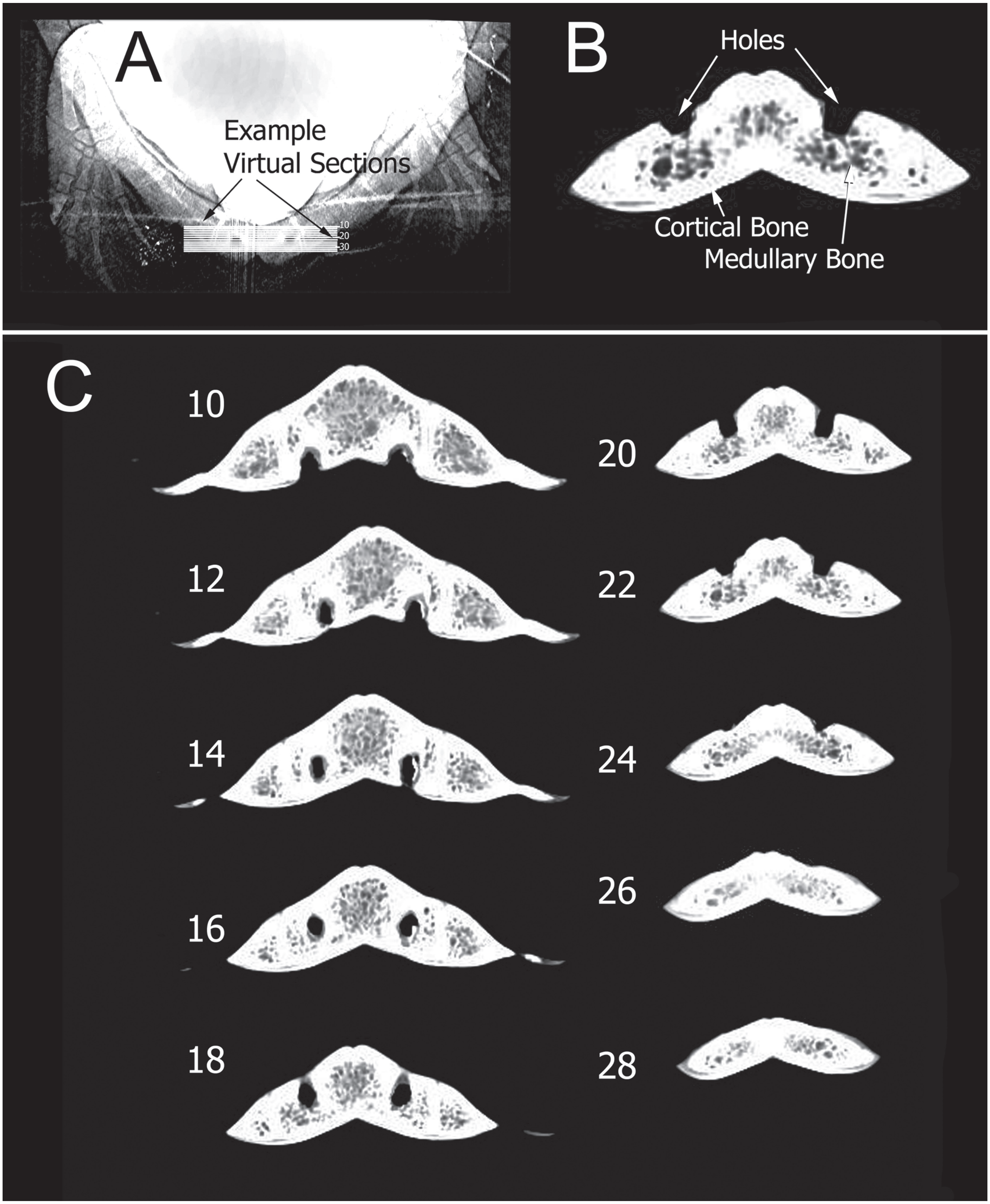

FIG. 4. (A) Computed tomography (CT) scans of the attachment site are collected as virtual serial sections (RRA806). (B) Cortical bone appears white; medullary (woven) bone appears gray. (C) Shown are the serial CT images of the posterior peripheral bones. Section 10 is the most anterior and section 28 the most posterior. The plane of sampling did not pass directly through the axis of PAT tag's mounting holes so they are shown starting with the inferior side of the holes and extending along the holes until just posterior to their superior openings. The bone along the holes is mostly compact, except along the tunnel walls where the stainless bolts passed (Images 12 right side, 14 right, 16-18). This gray material may represent adjacent debris or loosely woven bone. We could not distinguish the two without a biopsy; no biopsy was taken.

wall of webbing. During the first month all three turtles developed some microbial growth between the keratin and bone on the ventral surface around the holes. Over time, this resolved without treatment. Interestingly, it was least on the turtle that spent a month in Panama City where it was exposed to unfiltered sunlight.

After removing the tags, we found some cellular and keratinous debris in the bolt holes of two of the turtles, most of which fell out when the bolts were removed. Small depressions were present on the ventral surface around each hole, approximately the diameter of the washer. CT imaging showed the two bolt holes in the caudal-most shell region with little to no evidence of bone remodeling (Fig. 4C). None of the holes invaded into the coelomic cavity or caudal skin. There was no evidence of lysis surrounding the bolt holes and their borders had smooth margins; there was no evidence of clinically significant reaction.

The corrodible pins in the base of the tags were intact. The pin serves two purposes: (i) it is the weak link in the system and will break under enough stress, and (ii) it secures the tether to the tag and corrodes when the PAT-controlled application positively charges the pin to release the tag, which then will float to the surface. Temperature and salinity affect the rate of corrosion, but typically the pin breaks within 3 h, and is completely corroded within 9 h (Melinda Braun, Wildlife Computers, pers. comm.). Once the tag is free of the turtle, the turtle will still carry the tether and attachment hardware. Because the bolts were not marine grade stainless (e.g., they were 304 not 316 stainless), we fully expected the bolts to corrode and the hardware to be shed at some point well after PAT tag detachment on wild animals. There was some evidence of corrosion of the bolts, washers, and eyestrap when the hardware was removed after 1 yr.

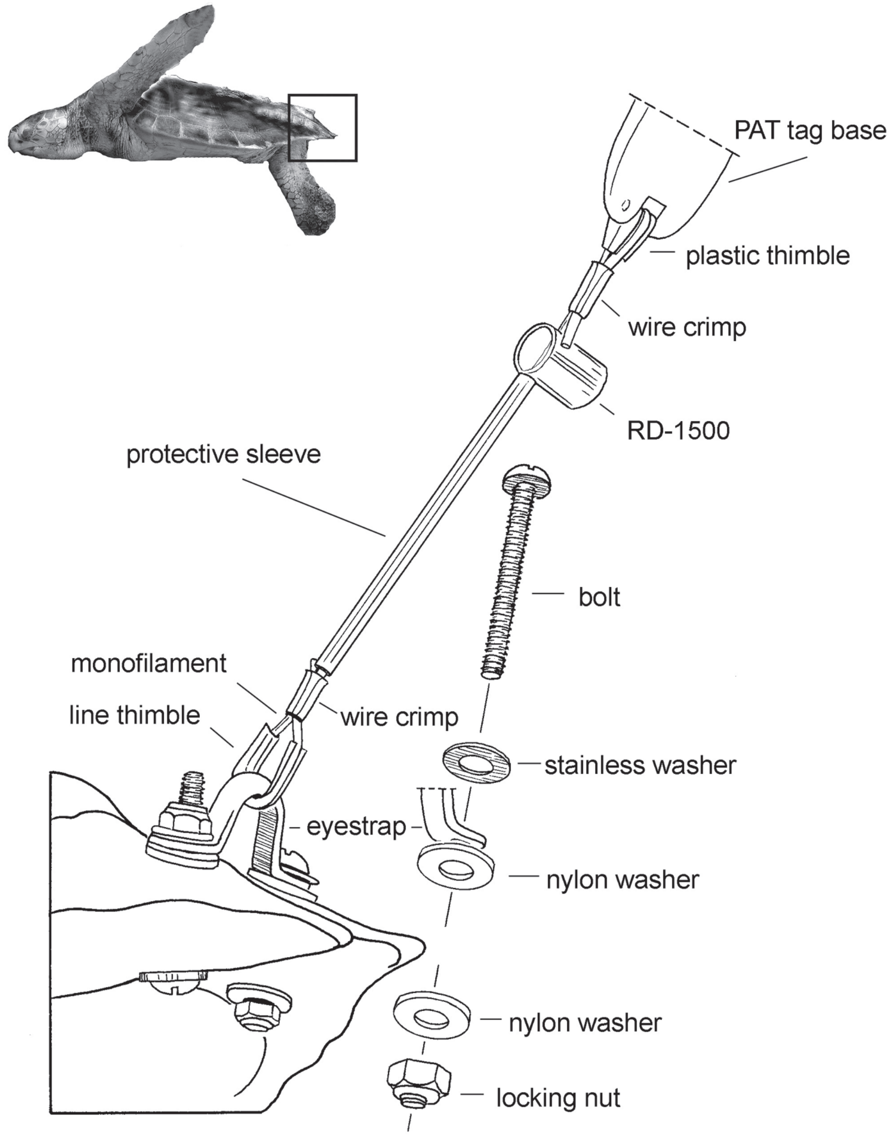

Fig. 5. Schematic of the current attachment hardware and tether for a PAT tag on a loggerhead sea turtle carapace (*Caretta caretta*). The corrodible breakaway pin in the base of the tag is not visible, but is surrounded by the plastic thimble. Note that the monofilament tail extending from the wire crimp sleeve is overlapping the RD-1500 to prevent it from spinning on the monofilament.

At the end of the study, the turtles were released in St. Joe Bay, Florida (29°49.5'N × 85°24.0'W) on 26 June 2004. They each bore 3 inconel flipper tags (one on the right front flipper and one on each rear flipper) and two 125 kHz passive integrated transponders (PIT tags; one in the right triceps superficialis muscle complex and one in the left front flipper, just above the second proximal scale on the trailing edge). Here we alert readers of the desire for follow-up information on the turtles, should anyone recapture the three test animals. We request readers to photograph the PAT tag attachment area (dorsal and ventral) and to contact us after the animal is released. Flipper tag numbers are RRA816/RRA801/RRA927 (PITs # 4367531D3B, 4349566D75), RRA806/RRA928/RRA929 (PITs # 43396C7F78, 4330691B6E), and RRA814/RRA930/RRA931 (PITs 4349504D1D, 43496A2344).

Since the conclusion of this laboratory study and after reviewing preliminary results from field testing, we made a few modifications to the tether and attachment to minimize the impact to the turtles and to ensure long deployments (Fig. 5). Wildlife Computers now recommends that the RD-1500 be placed adjacent to the corrodible pin in the tag and that a small length of the monofilament tether be left exposed through the crimp sleeve on the tether, overlapping the RD-1500 to prevent it from spinning around the monofilament which could score and weaken the line. Additionally, we learned that the application of heat weakens monofilament and no longer use heat shrink tubing. Instead, we now protect the monofilament with a flexible translucent sleeve (1.85 mm internal diameter). We found a variety of monofilament lines of the same diameter (1.8 mm), ranging in strength from 136 kg-test to 182 kg-test, and we use them interchangeably. As a direct result of this laboratory research we now are using larger diameter (13 mm O.D.) nylon washers placed adjacent to the plastron and the carapace, and a 12 mm #10 stainless washer between the eye strap and the head of the bolt or the lock nut. Lastly, we are ensuring that the nuts are not over-tightened to minimize pressure necrosis.

We conclude that this procedure can be used in the field to attach PAT tags to wild cheloniid turtles of all species (with the possible exception of *Natator depressus*) without detrimental effect on the turtle, and that the attachment would be useful for long term tag deployments. Using the described methodology, NOAA Fisheries Service has released PAT tags on pelagic oceanic loggerheads captured in the north central North Atlantic Ocean. Of the 19 tags programmed for long deployments and that transmitted, only 4 released prior to 6 months (Sasso and Epperly 2007); we do not believe their premature release was due to attachment or tether failure.

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